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## Regulation of cytokines' level in the oral liquid of young patients with the generalized periodontitis in the use of different ways of treatment

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### Abstract

There were examined and treated 37 somatically healthy persons of young age (18-25 years) with generalized periodontitis (GP) of initial-I degree of development, who were included into 2 groups: group I – 20 patients with chronic generalized periodontitis (CGP) and group II – 17 patients with exacerbation of chronic generalized periodontitis (ECGP). Control was provided by 8 practically healthy persons with a healthy oral cavity. For treatment, patients were divided into subgroups: the main (IA – 10 people and IIA – 9 people) and control ones (IB – 10 people and IIB – 8 people). The content of interleukin-18 (IL-18) and interleukin-10 (IL-10) in the oral liquid before and after the complex treatment was determined. There was a significant imbalance in the local cellular and humoral immunity, as evidenced by a significant increase of IL-18 and a decrease in the content of IL-10, especially in ECGP. Under the influence of complex therapy, the method developed by us there was a significant regulation of the cytokine spectrum: the level of IL-18 decreased, and IL-10 – increased with the achievement of data in healthy persons. The regulation of immune reactivity with the help of herbal immunomodulators has shown the greater efficiency and prospectivity of the therapeutic complex developed by us, than the use of synthetic medicines used for this purpose, and it has no negative consequences for the health of young people.

**Keywords:** Generalized periodontitis, oral fluid, interleukin-18, interleukin-10, complex therapy, herbal preparations

### Introduction

Cytokines – are the protein-peptide factors that are produced by the cells, regulate intercellular and intersystem interaction (cell survival, stimulation and inhibition of their growth). According to the biologic effect cytokines are conventionally divided into pro-inflammatory and anti-inflammatory, and according to their functions – type 1 cytokines involved into the regulation of cellular immunity (IL-1 $\beta$ , IL-2, IL-12, IL-15, IL-18, TNF- $\alpha$ ), and type 2 cytokines that stimulate humoral immunity and weaken cellular one (IL-4, IL-5, IL-6, IL-10, IL-13) [3, 4, 14].

The role of cytokines in the mechanisms of development of chronic inflammation in periodontal tissues, along with other concepts of the pathogenesis of generalized periodontitis (GP), has been determined, in which they play a leading role in the development of continuous inflammation in the periodontium induced by microbial agents. In this case, the periodontal-pathogenic microflora acts as a trigger mechanism in the pathological activation of periodontal macrophages and in their induction of a cascade of pro-inflammatory cytokines, which, in turn, causes periodontal damage and resorption of bone tissue [2, 6, 8, 10]. Imbalance in the system of cytokines in GP is manifested by the prevalence of pro-inflammatory over anti-inflammatory ones, which violates the interactions in the local system and can lead to pathology of regeneration of granulations, damage to bone regeneration of the alveolar appendix and the formation of periodontal pocket [1, 9].

In recent years, IL-18 is considered to be an important cytokine in the progression of periodontal disease [15, 16]. Its higher level in gums and oral fluid in GP was noted in comparison with healthy persons and patients with gingivitis. In some studies, the increase in the content of IL-18 in the oral liquid of patients with GP was determined only with its exacerbation [5, 13], which may be indicative of the activation of monocytes/macrophages and dendritic cells, which are the main source of this cytokine, in response to microbial aggression [5]. As an anti-inflammatory cytokine, IL-10 plays an important role in the progression of periodontal diseases.

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Its presence suppresses the inflammatory reactions, and its absence leads to the increased resorption of alveolar bone and reduces bone tissue formation as it has a suppressive effect on production of IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , G-CSF, etc. A number of modern studies indicate that the polymorphism of this cytokine gene is an important component of the GP development [17, 18]. It was determined that in patients with GP, the level of IL-10 is significantly lowered, especially in case of I and II degrees of development [7].

Today, the research on pro- and anti-inflammatory cytokines continues, therefore, we believe that the study of the dynamics of IL-10 and IL-18 indices in young patients with GP under the influence of complex treatment remains relevant.

The purpose of the research is to study the changes of cytokines' level in the oral liquid in patients with chronic generalized periodontitis (CGP) and exacerbation of chronic generalized periodontitis (ECGP) and to compare the effects of the treatment complex developed by us and the generally accepted therapy on all of these indicators.

**Material and methods of research:** There were examined and treated 37 somatically healthy young patients (18-25 years old) with GP of initial-I degree of the development. They were divided into 2 groups: group I – 20 patients with CGP and group II – 17 patients with ECGP. Control was provided by 8 practically healthy persons with a healthy oral cavity. For treatment, the patients were divided into subgroups: the main (IA – 10 people and IIA – 9 people) and control ones (IB – 10 people and IIB – 8 people).

The level of IL-10 and IL-18 in the oral liquid before and after the treatment was studied using enzyme-linked immunosorbent assay using a commercial kit of the firm "Vektor BEST" (Russia), the immune enzyme analyzer "Sunrise" (Tecan, Austria), according to the instructions of the manufacturers. The oral fluid was taken in the morning, in the fasted state, without oral hygiene into the sterile test glasses, frozen and stored for the research in this state at a temperature of – 20°C... – 25 °C.

The patients with GP from the main subgroups were used the method of complex treatment developed by us, in which, after the initial periodontal therapy, oral dishes were administered of a standard solution of St. John's wort tincture twice a day during 5-7 days and applications onto the gums and instillations of the gel developed by us into the periodontal pockets (it consists of: tincture of Echinacea Purpurea – 1 ml; tincture of St. John's wort – 1 ml; Enterosgel – 2 g) during 20-30 minutes once a day, for 5-7 days [11]. The syrup "Immuno-tone" (based on tincture of Echinacea Purpurea and St. John's wort and Eleutherococcus) was prescribed per os 2-3 teaspoons (10-15 ml) 2 times a day, the course – 10 days. [12]

The patients with GP of the control subgroups were used the general treatment: locally – initial periodontal therapy, rinsing with 0.05% solution of chlorhexidine bigluconate twice daily, during 5-7 days, application and instillation of the "Metrogyl-denta" gel for 15-20 minutes once per day, during 5-7 days, multivitamins per os, course – 10 days [11].

**Results of the research and their discussion:** It was found by us that the level of interleukins in the oral liquid of patients with CGP of the initial-I degree of development varied (Table 1). In this case, in group I the content of pro-inflammatory cytokine IL-18 of the main and control subgroups differed

from the indicators in healthy ones: it was 1.43- and 1.31-fold higher, however, there was no significant difference between the indexes of norm and patients. At the same time the amount of IL-10 in the oral liquid of patients with CGP of the IA and IB subgroups was significantly less than in the healthy ones – 2.05- and 2.19-fold ( $p < 0.001$ ) respectively. There was no significant difference between the subgroups IA and IB according to both indicators.

In ECGP (group II), the titers of IL-18 in the oral liquid of IIA subgroup grew 1.77-fold ( $p < 0.05$ ), and in IB – 1.69-fold ( $p < 0.05$ ) compared with the data of healthy, as it is shown in Table 2. At the same time, the titers of IL-10 were lower than the reference values in healthy persons 3.38- and 3.47-fold ( $p < 0.001$ ) in the IIA and IIB subgroups respectively. Indicators of both subgroups of group II according to data on the content of IL-18 and IL-10 were close ( $p > 0.05$ ).

In order to determine the differences of the content of pro- and anti-inflammatory interleukins in the oral fluid of patients with CGP and ECGP, we compared them among patients in the main subgroups. It was found that the level of IL-18 in the case of exacerbation of the pathological process in periodontium (IIA subgroup) was 24.15% higher than in the chronic course of the disease (IA subgroup), but the difference was unreliable. At the same time, the concentration of IL-10 in the oral liquid of patients with ECGP was 53.56% lower than in CGP, and the difference was significant ( $p < 0.05$ ).

After the performed by us measures for our patients in the main and control subgroups of groups I and II, the changes in the level of cytokines occurred in the oral liquid. Thus, in patients with CGP of the IA subgroup, the content of IL-18 in the oral liquid decreased 1.73-fold ( $p_1 < 0.05$ ): from (398.98 $\pm$ 43.33) pg/ml to (230.93 $\pm$ 33.80) pg/ml, thus, it became lower than in healthy ones, and the difference with the data of healthy ones became insignificant (Table 3). At the same time, we noted an increase of the IL-10 level in the oral liquid of patients of the IA subgroup while using the medicine "Immuno-tone": the concentration of this cytokine increased 1.59-fold: from (12.97 $\pm$ 1.76) pg/ml to (20.58 $\pm$ 2.32) pg/ml ( $p_1 < 0.05$ ), approaching to data in healthy, and probably did not differ from their indicators ( $p_2 > 0.05$ ).

In patients with ECGP of the IIA subgroup the number of cytokines in the oral fluid under the influence of treatment has also changed. In particular, the content of IL-18 decreased 1.71-fold ( $p_1 < 0.001$ ): from (495.33 $\pm$ 32.76) pg/ml to (290.24 $\pm$ 38.60) pg/ml ( $p_1 < 0.001$ ) and was close to the values in healthy ( $p_2 > 0.05$ ). At the same time, the level of IL-10 in ECGP as a result of complex treatment increased 1.62-fold ( $p_1 < 0.05$ ): from (7.86 $\pm$ 1.56) pg/ml to (12.78 $\pm$ 1.33) pg/ml, but the difference with the data of healthy was significant ( $p_2 < 0.001$ ), making 2.07-fold.

In CGP patients' treatment of the IB subgroup where there was used traditional therapy, changes in the level of cytokines in the oral liquid were not so pronounced (Table 4). In particular, the titer IL-18 decreased 1.19-fold ( $p_1 > 0.05$ ): (367.48 $\pm$ 31.97) pg/ml to (309.13 $\pm$ 22.15) pg/ml, and became close to the data in healthy individuals; the difference with them was 1.11-fold ( $p_2 > 0.05$ ). At the same time the titer of IL-10 has increased 1.25-fold: from (12.10 $\pm$ 1.13) pg/ml to (14.54 $\pm$ 0.96) pg/ml ( $p_1 > 0.05$ ), which did not predetermine its normalization, because the difference with the healthy ones was 1.75-fold ( $p_2 < 0.05$ ).

Traditional treatment in case of ECGP also led to a significant decrease in the titers of IL-18 in the oral fluid of young people

of the IIB subgroup – 1.47-fold ( $p_1 < 0.05$ ): from  $(474.85 \pm 24.99)$  pg/ml to  $(322.23 \pm 43.50)$  pg/ml. However, the concentration of IL-10 increased slightly – 1.27-fold: from  $(7.65 \pm 1.58)$  pg/ml to  $(9.73 \pm 1.89)$  pg/ml ( $p_1 > 0.05$ ) and did not cause its normalization, because the difference with the healthy ones was reliable ( $p_2 < 0.001$ ).

In order to find out the differences between the data of patients in the main and control subgroups after the complex treatment, they were compared by us and we have found, that despite the fact that the difference in the obtained indices of the amount of IL-18 in the oral fluid between the IA and IB subgroups was 33.86% in favor of the IA subgroup, and between the IIA and IIB subgroups – 11.02% in favor of the IIA subgroup, it was unreliable. Due to the content of IL-10, the difference between its indicators in the IA and IB subgroups was 41.35% in favor of the IA subgroup and was probable ( $p < 0.05$ ), but between the data of the IIA and IIB subgroups the difference of 31.35% in favor of the IIA subgroup was unconvincing. This is explained by the small amount of sampling and the large variations of the numbers in the variation ranges of the comparable subgroups.

The results of our study showed an increase of the content of IL-18 in the oral liquid of patients with GP in both variants of its course, but much more – in its exacerbation. The amount of IL-10 is significantly reduced in all patients, and especially – in case of ECGP, as well as the elevated level of IL-18

indicate a marked violation of the cytokine spectrum and strain in the local cellular and humoral immunity.

Due to the complex treatment of GP, irrespective of our chosen method, the elimination of the infectious factor is eliminated and the inflammatory immune response is balanced, which is manifested by a decrease of the level of pro-inflammatory IL-18 and an increase of the content of anti-inflammatory IL-10 in the oral fluid of patients with CGP and ECGP of young people. This is the evidence of activation of both the cellular and humoral link of local immunity in the direction of elimination of ethiopathological factors involved in the onset and development of a GP.

Comprehensive treatment of patients with GP, using the method developed by us, contributed to a greater regulation of the cytokines' content in the oral fluid, apparently due to the fact that "Immuno-tone" has typical immunomodulatory properties: inhibits the excessive inflammation by reducing the formation of IL-18 and increasing the production of IL-10, thus inhibiting the processes of destruction of periodontal tissues, especially in CGP. The regulation of local cellular and humoral immunity with the help of herbal means promotes the rapid obtaining of positive result after periodontal treatment, does not cause undesirable side effects, as in case of treatment with antibacterial drugs of chemical origin, and causes an indirect antibacterial effect by mobilizing its own protective forces of the organism.

**Table 1:** Content of IL-18 and IL-10 in the oral liquid of patients with chronic generalized periodontitis (group I) of initial-I degree of development ( $M \pm m$ )

Indexes	Healthy	Patients with chronic generalized periodontitis (group I)	
		main subgroup (IA)	control subgroup (IB)
IL-18, pg/ml	n=8 279.35±42.11	n=10 398.98±43.33	n=10 367.48±31.97
IL-10, pg/ml	n=8 26.56±2.73	n=10 12.97±1.76 ***	n=10 12.10±1.06 ***

**Table 2:** Content of IL-18 and IL-10 in the oral liquid of patients with acute generalized periodontitis (II group) of initial-I degree of development ( $M \pm m$ )

Indexes	Healthy	Patients with chronic generalized periodontitis (group II)	
		main subgroup (IIA)	control subgroup (IIB)
Показники	Здорові	Хворі на загострення хронічного генералізованого пародонтиту (II група)	
		основна підгрупа (IIA)	контрольна підгрупа (IIB)
IL-18, pg/ml	n=8 279.35±42.11	n=9 495.33±32.76 *	n=8 474.85±24.99 *
IL-10, pg/ml	n=8 26.56±2.73	n=8 7.86±1.56 ***	n=8 7.65±1.58 ***

**Table 3:** The dynamics of the indexes of the content of IL-18 and IL-10 in the oral liquid of patients with generalized periodontitis of the initial-I degree of development under the influence of the complex therapy developed by us in case of chronic (group I) and acute (group II) course of the disease ( $M \pm m$ )

Indexes	Healthy	Patients with chronic generalized periodontitis (IA subgroup)		Patients with exacerbation of chronic generalized periodontitis (IIA subgroup)	
		before treatment	immediately after treatment	before treatment	immediately after treatment
IL-18, pg/ml	n=8 279.35±42.11	n=10 398.98±43.33	n=10 230.93±33.80 *	n=9 495.33±32.76 #	n=9 290.24±38.60 ***
IL-10, pg/ml	n=8 26.56±2.73	n=10 12.97±1.76 ###	n=10 20.58±2.32 *	n=8 7.86±1.56 ###	n=8 12.78±1.33 * ###

**Table 4:** The dynamics of the indexes of the content of IL-18 and IL-10 in the oral liquid of patients with generalized periodontitis of the initial-I degree of development under the influence of conventional complex therapy in case of chronic (group I) and acute (group II) course of the disease (M±m)

Indexes	Healthy	Patients with chronic generalized periodontitis (IB subgroup)		Patients with exacerbation of chronic generalized periodontitis (IIB subgroup)	
		before treatment	immediately after treatment	before treatment	immediately after treatment
IL-18, pg/ml	n=8 279.35±42.11	n=10 367.48±31.97	n=10 309.13±22.15	n=8 474.85±24.99 #	n=8 322.23±43.50 *
IL-10, pg/ml	n=8 26.56±2.73	n=10 12.10±1.13 ###	n=10 14.54±0.96 #	n=8 7.65±1.58 ###	n=8 9.73±1.89 ###

Note. The probability of difference of indexes is indicated: \* –  $p_1 < 0.05$  – the level of reliability of the values in comparison of the data received immediately after the treatment, with the indicators received before the treatment; # –  $p_2 < 0.05$ ; ### –  $p_2 < 0.001$  – the level of the probability of values in comparison of the data obtained after the treatment, with the indicators of healthy.

**Conclusions:** 1. Study of the amount of IL-18 and IL-10 in the oral liquid of young patients with CGP and ECGP showed a significant imbalance in local cellular and humoral immunity, as evidenced by a significant increase of IL-18 level and a decrease in the content of IL-10, especially in ECGP. 2. Under the influence of complex therapy with the method developed by us, the significant regulation of the cytokine spectrum has occurred: the level of IL-18 decreased 1.73-fold ( $p_1 < 0.05$ ) and 1.71-fold ( $p_1 < 0.001$ ), and IL-10 – elevated 1.59- and 1.62-fold ( $p_1 < 0.05$ ) in the IA and IIA subgroups, respectively, with the achievement of healthy data. 3. Treatment of patients in the traditional way also contributed to some regulation of cytokines in the oral liquid in the IB and IIB subgroups, but significantly lowered (1.47-fold,  $p_1 < 0.05$ ) and only IL-18 index was close to normal. 4. Regulation of immune reactivity and maintaining it at an appropriate level with the help of herbal immunomodulators is more effective and promising than the use of synthetic medicines for this purpose, and has no negative consequences for the health of young people.

The prospect of further research is the study of changes in other indices under the influence of complex treatment of young patients with GP.

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